

Fig. 1A

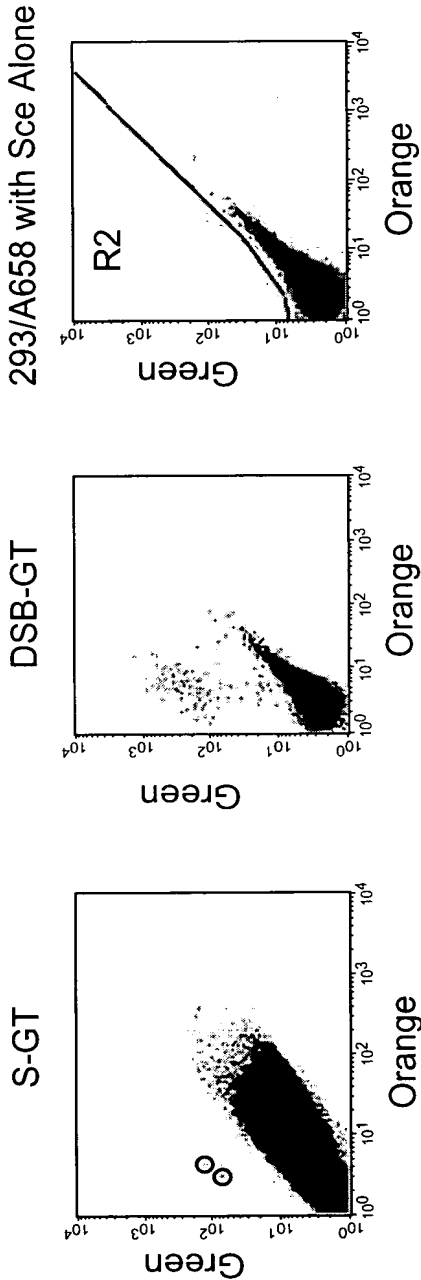


Fig. 1B

Gene Target	1bp insertion		7 bp insertion		35 bp insertion (A658)		35 bp insertion (A658)		66 bp insertion (QQR8)		Fold Stimulation By Sce Induced DSB
	Sce	No	No	No	No	Yes	No	Yes	Yes		
Events/ 10 ⁶ cells		29.5 +/- 13.0	1.5 +/- 1.1	0.71 +/- 0.27		1600 +/- 500			1900 +/- 250		>2000
Rate		2.9 x 10 ⁻⁵	1.5 x 10 ⁻⁶	7.1 x 10 ⁻⁷		1.6 x 10 ⁻³			1.9 x 10 ⁻³		

Fig. 1C

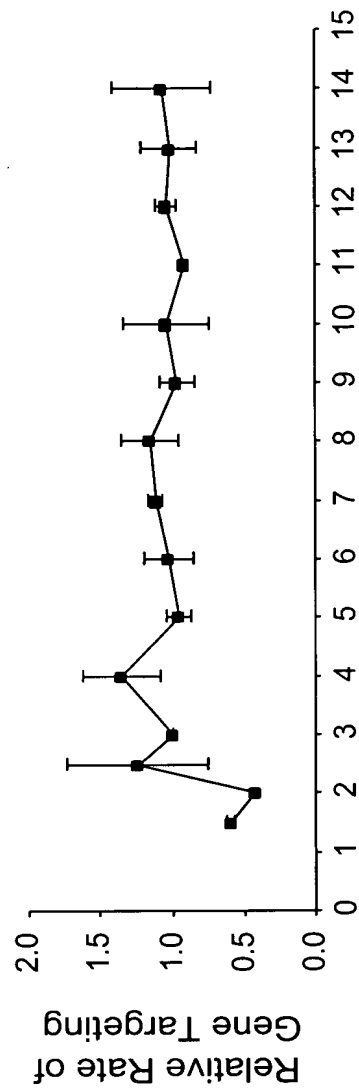


Fig. 1D

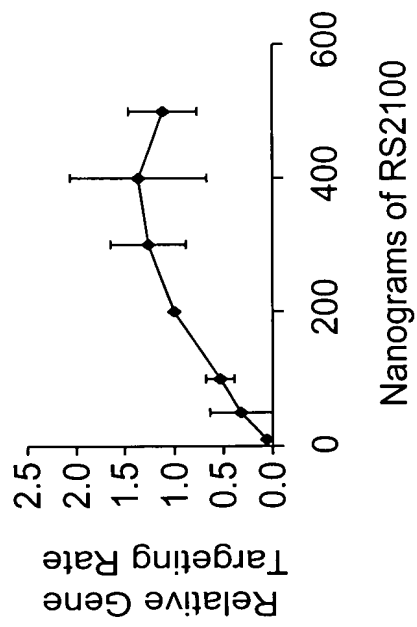


Fig. 2A

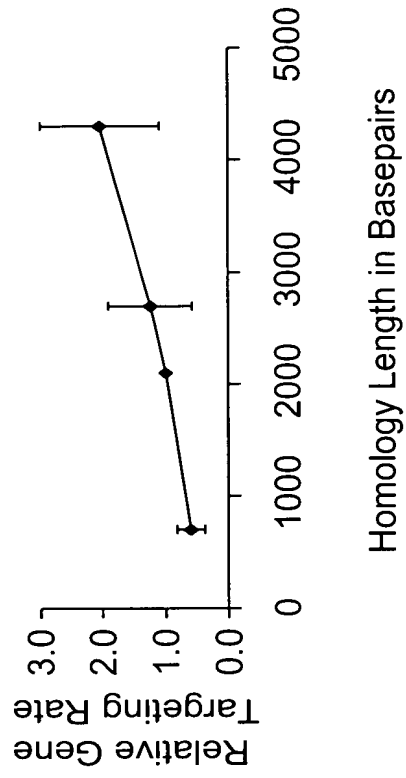


Fig. 2B

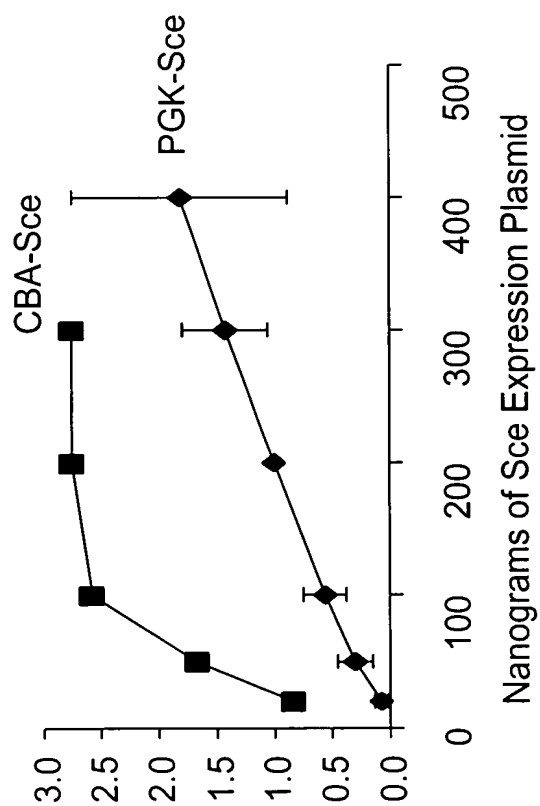


Fig. 2C

Fig. 2D

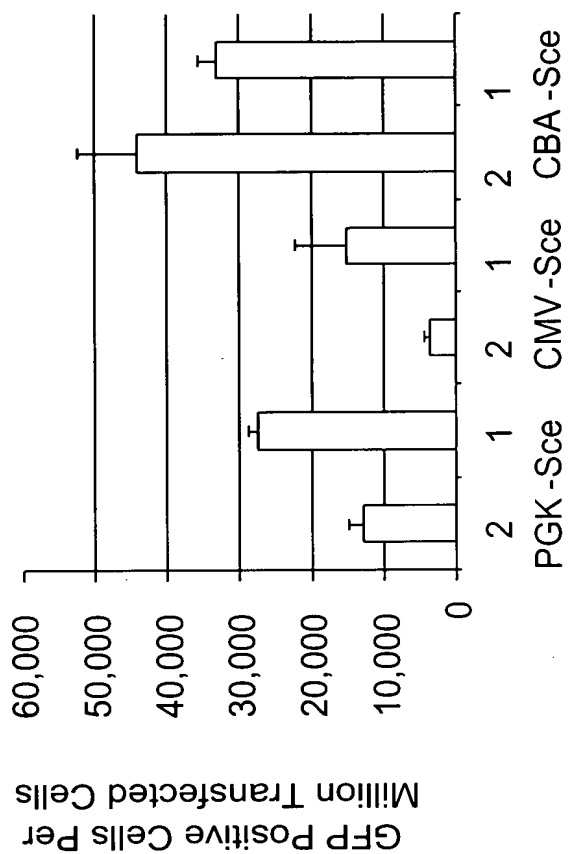
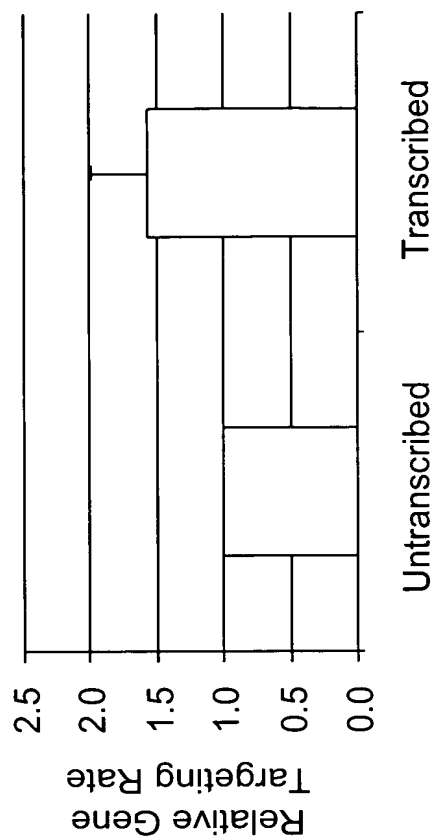


Fig. 2E

Targets:

QQR8: 5' GFP Stop QQR Site 8 8 11 S R 0 0 Sc e Site 3' GFP
 QQR6: 5' GFP Stop QQR Site 6 6 11 S R 0 0 Sc e Site 3' GFP
 QQRZif6: 5' GFP Stop QQR Site 6 6 11 Z 11 S Sc e Site 3' GFP

Chimeric Nucleases:

CMV-QQR-L18-Fn: CMV ATG N QQR L 18 Nuclease
 CMV-QQR-L0-Fn: CMV ATG N QQR L 0 Nuclease
 CMV-Zif-L3-Fn: CMV ATG N Zif L 3 Nuclease

Fig. 3A

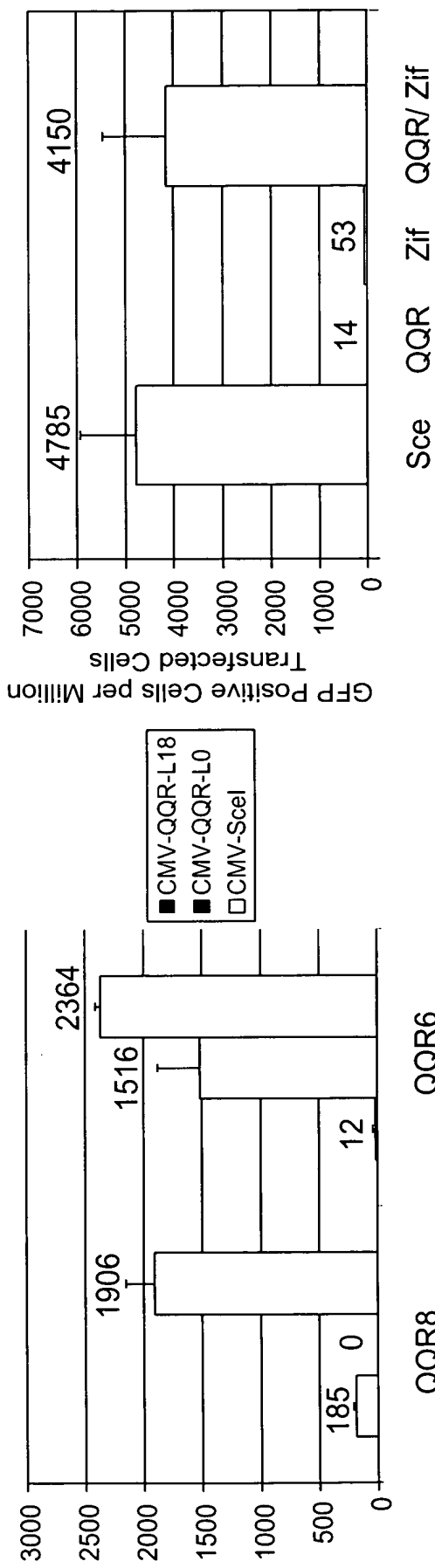


Fig. 3B

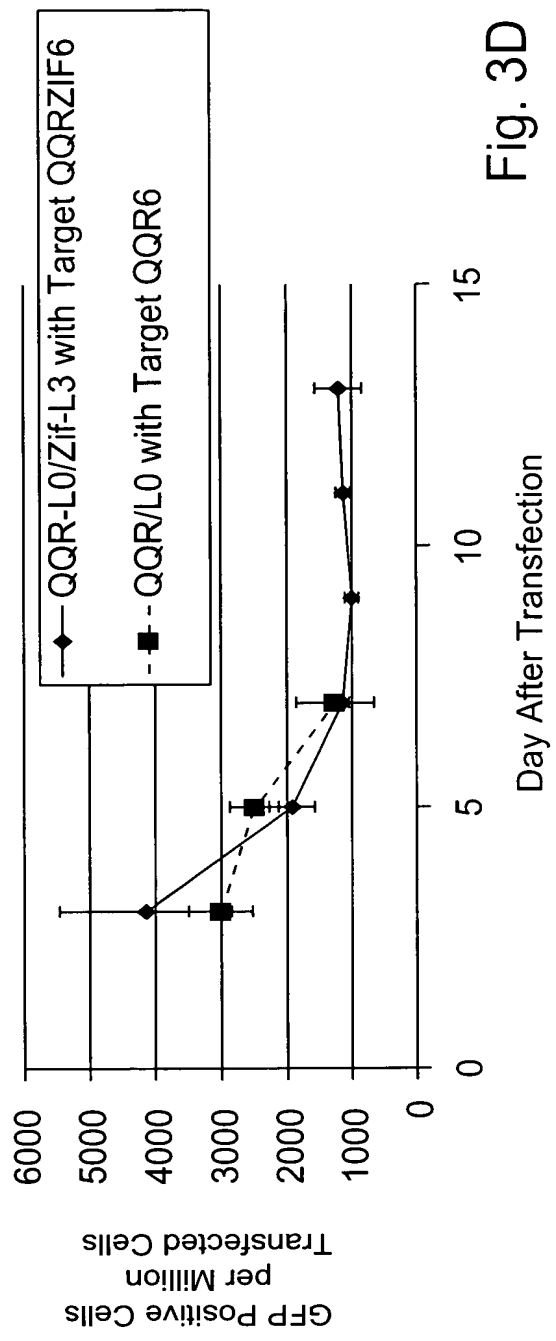


Fig. 3D

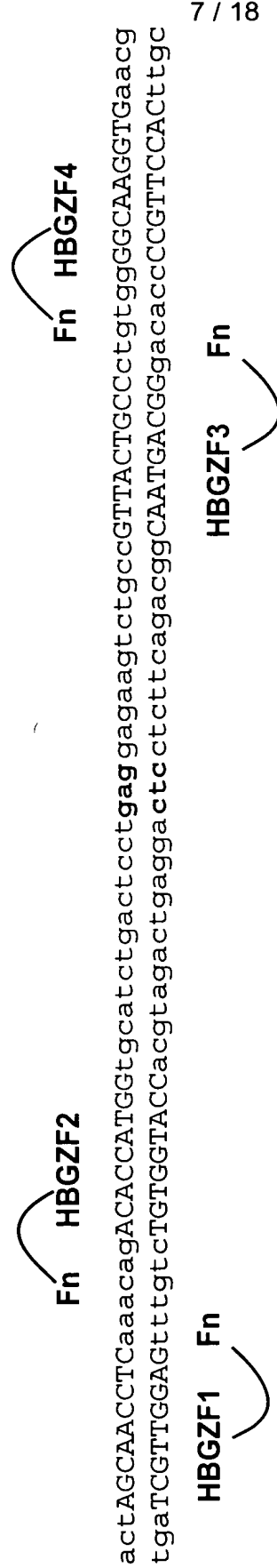


Fig. 4

Design of HBGZF1

Target Binding Site: 5' GAG GTT GCT 3'
F3 F2 F1

-1 1 2 3 4 5 6
B3 B2 B1

Finger 1: GCT Q S S D L T R*
T S G E L V R

Finger 2: GTT T S G A L T R*
T S G S L T R

Finger 3: GAG R S D N L T R*
R S D N L V R

Structure of CMV - HBGZF1

CMV ATG - NLS - FLAG - Finger 1(QSSDLTR) - Finger2(TSGALTR) - Finger3(RSDNLTR) - Fn

Fig. 5

Gene Targeting with HBGZF1

Target: GFP-Stop-AGCAACCTCtctagaGCGTGGCG-SceSite-GFP
TCGTTGGAGagatctCGCACCCGC

HBGZF1 Fn ZIF268

HBGZF1 Fn

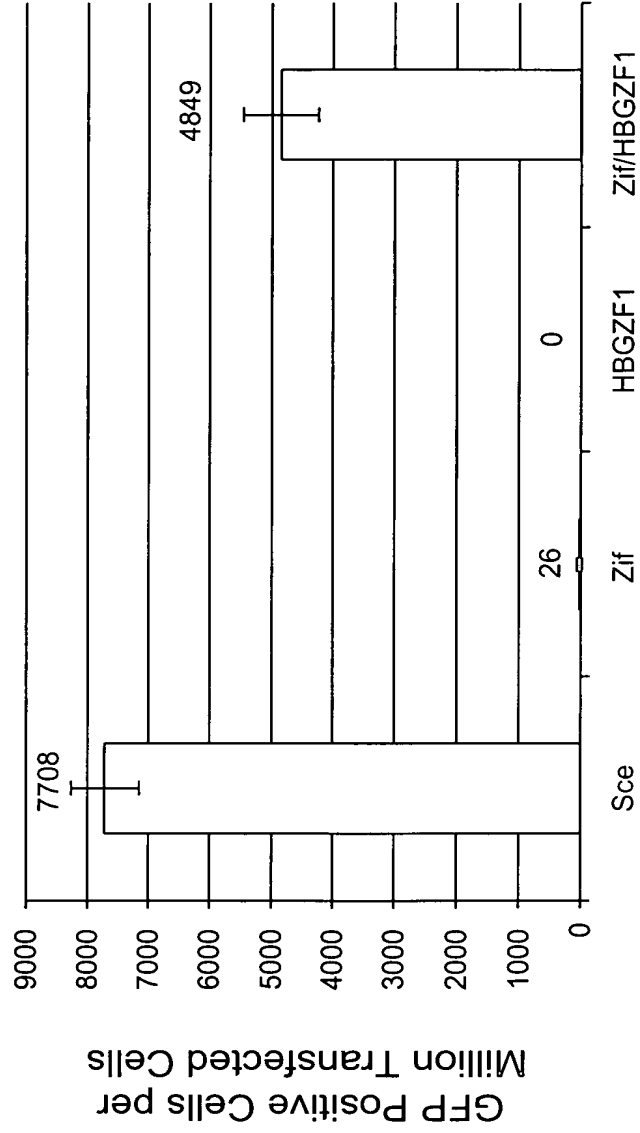


Fig. 6

Design of HBGZF4

Target Binding Site: 5' GGC AAG GTG 3'
F3 F2 F1

-1 1 2 3 4 5 6
B3 B2 B1

Finger 1: GTG R S D A L T R*
R S N S L T R

Finger 2: AAG R S D T L S N
R K D N L K N
R S S N L T Q*

Finger 3: GGC D R S H L A R*
E S N H L T R
E R S K L A R
D P G H L V R

Structure of CMV-HBGZF1

CMV ATG-NLS-FLAG-Finger 1(RSDALTR)-Finger2(RSSNLTQ)-Finger3(DRSHLAR)-Fn

Fig. 7

Gene Targeting with HBGZF4

Fn ZIF268

Target: GFP-Stop-CACCTTGCCtctagaGCGTGGCG-SceSiteGFP
GTGGAACGGagatctCGCACCCGC

HBGZF4 Fn

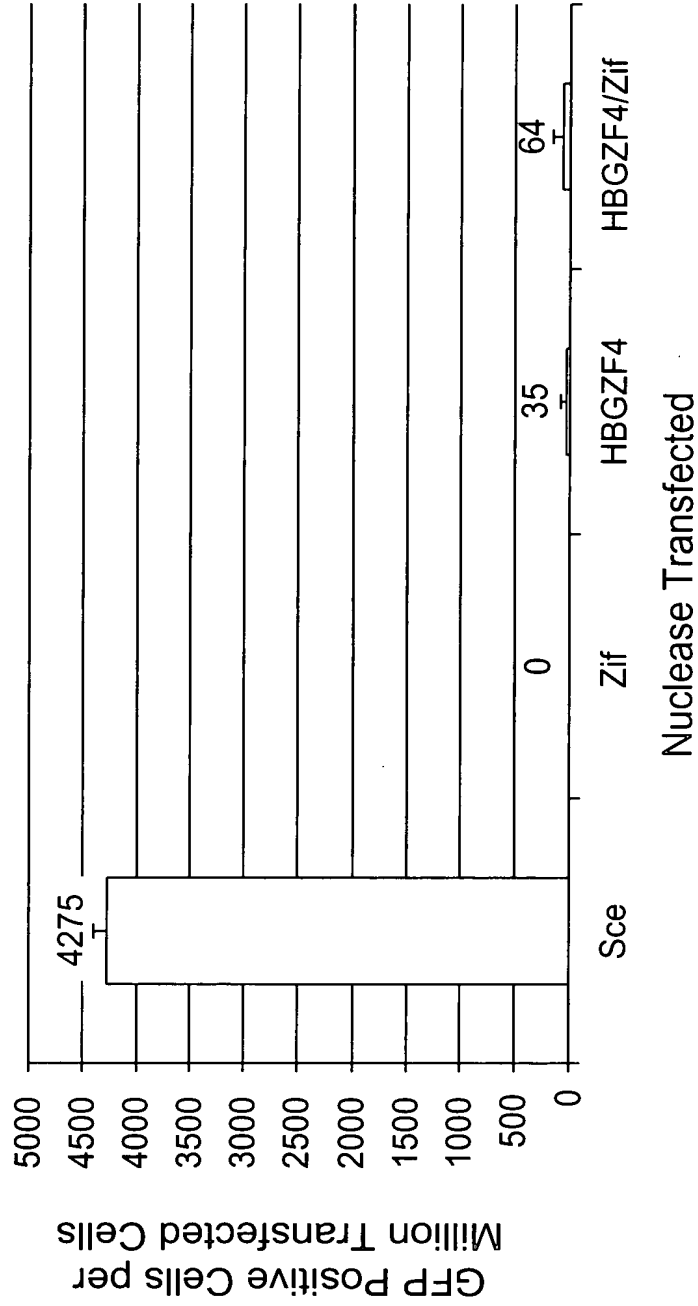


Fig. 8

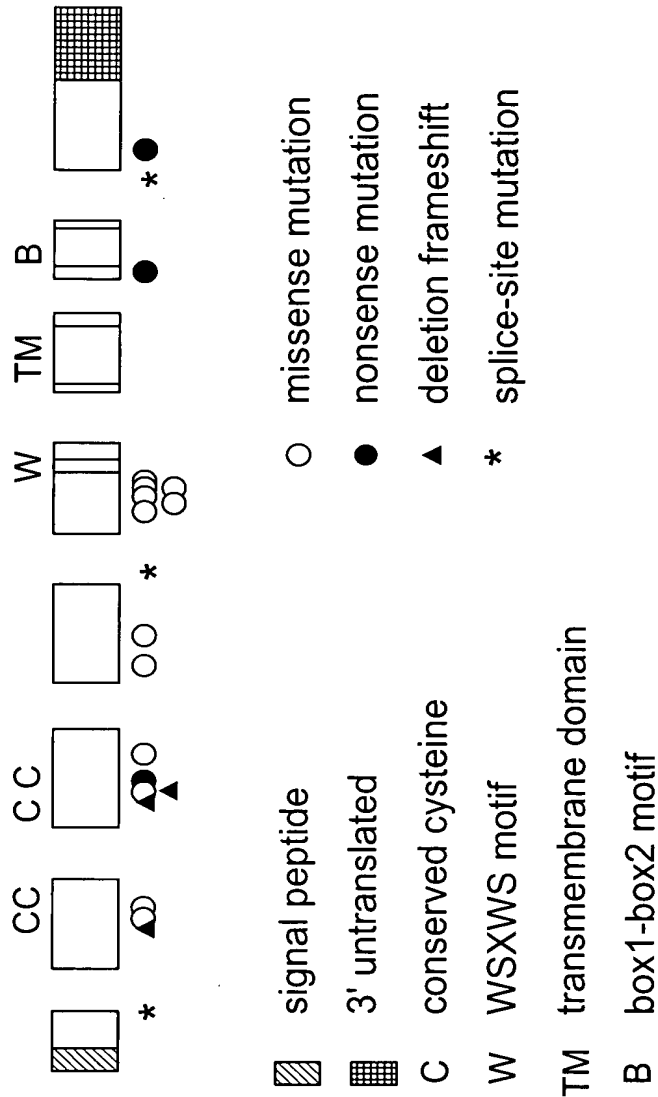


Fig. 9A

Exon 5 5' gaacaatcagtgattatagacataaagtctccttgccctagtggtggatgggcagaaacgctacacgttctcgtgtt
3' cttgttagtcacctaatatctgtattcaagaggaaacggatcacacctaccgctcttgcgatgtgaaagcaca
cggagccgctttaaccactctgtggaagtgcctcagcat **WSXWS motif** **HCGCZF2**
gcctcggcgaaattgggtgagacaccttcacgagtcgtaacctcacttacctcgggtgggttaggtgacccccctcgt
atacttcaaaagag 3' **HCGCZF1**
tatgaagttttctg 5'

Fig. 9B

Design of HCGCZF2

Target Binding Site: 5' GGG GGA GCA 3'

F3 F2 F1

-1 1 2 3 4 5 6
B3 B2 B1

Finger 1:	GCA	Q	S	G	S	L	T	R
		Q	S	G	D	L	T	R
		Q	S	G	D	L	T	R
		Q	S	N	D	L	T	R*
Finger 2:	GGA	Q	S	G	H	L	Q	K
		Q	R	A	H	L	E	R
		Q	S	S	H	L	T	R*
Finger 3:	GGG	R	S	D	H	L	A	R
		R	S	D	H	L	T	R
		R	S	S	H	L	T	R*

Structure of CMV-HCGCZF2

CMV

ATG-NLS-FLAG-Finger 1(QSNDLTR)-Finger2(QSSHLTR)-Finger3(RSSHLTR)-Fn

Fig. 10

Gene Targeting with HCGCZF2

Fn ZIF268

Target: GFP-Stop-TGCTCCCCCtctagaGCGTGGGCG-SceSite-GFP
ACGAGGGGgagatctCGCACCCGC

HCGCZF2 Fn

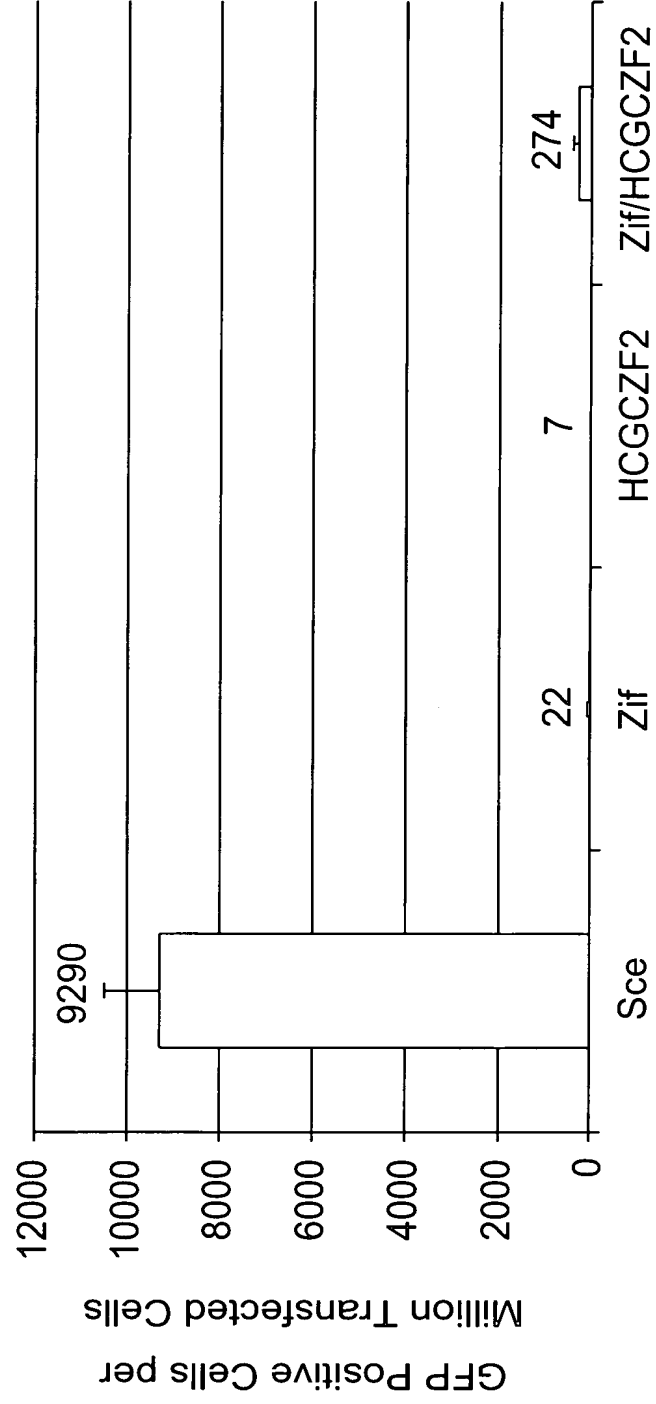


Fig. 11

Fn  **GFPCN2**

5'acC atC ttc ttcaag Gac Gac Ggc aac stop-Sce site tac
3'tgG taG aag ttc Cgc Ctg ccg ttc


GFPCN1  **Fn**

Fig. 12A

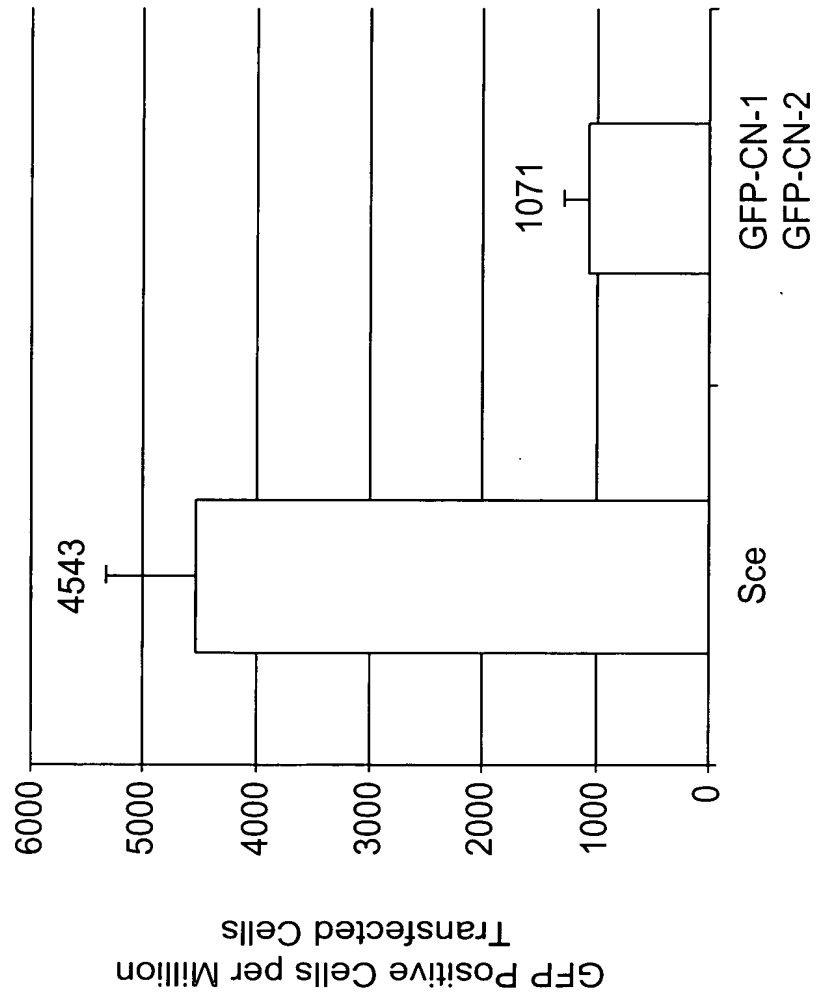


Fig. 12B

Fn  **CD8ZF2**

bp 441 5' acc ggCgcCcaC catcg GtcG caGcc ctg3' bp 471
 tgg ccG cgGgtG gtagcg cagCgtCgg gac

CD8ZF1  **Fn**

Fig. 13A

Cell Line 293/1104	CMV/CBA	GFP*	IRES	CD8	95% CD8 Positive
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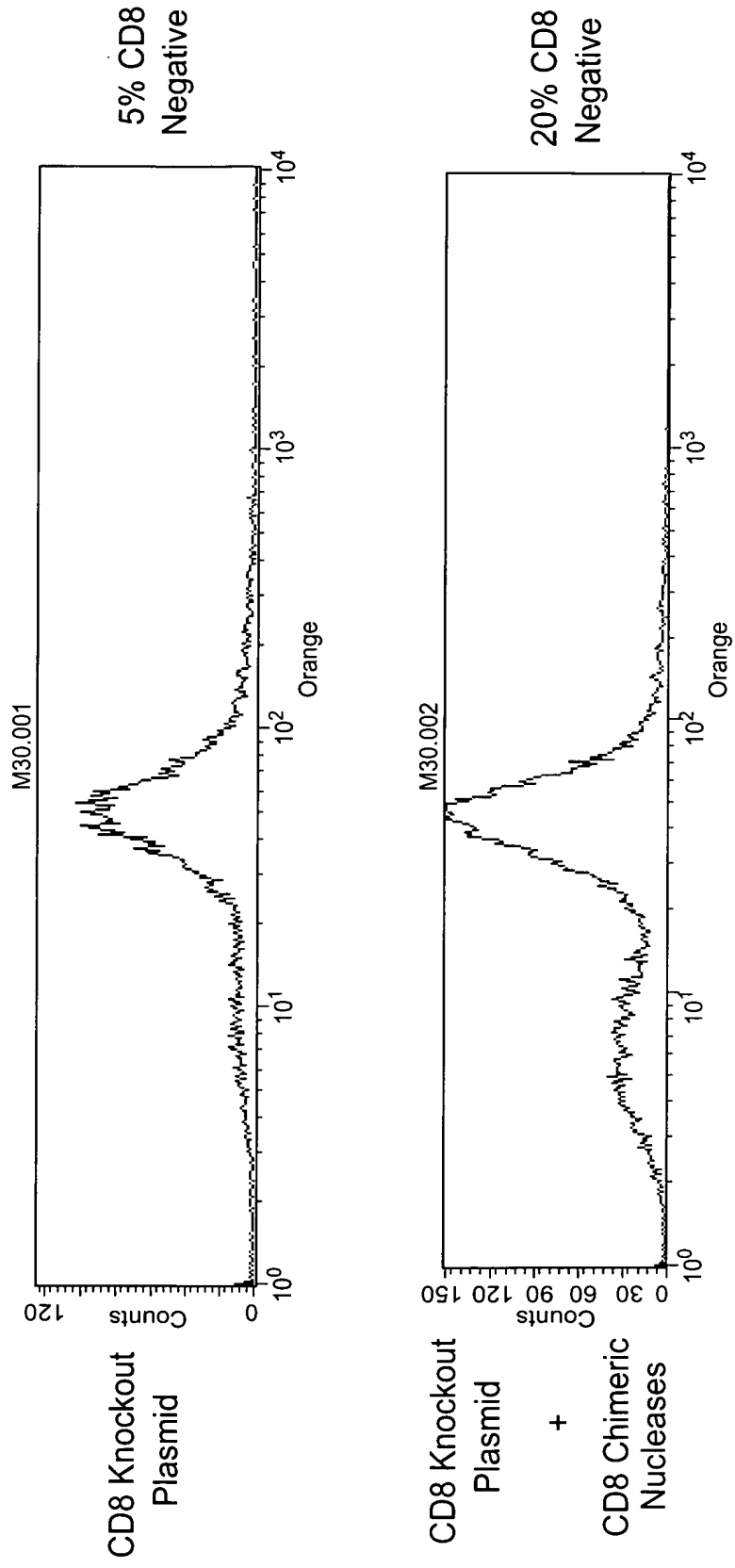


Fig. 13B